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Coagulation parameters predictive of polycystic ovary syndrome

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ABSTRACT

Objective: To explore coagulation parameters in association with polycystic ovarian syndrome (PCOS) and establish a model for predicting the risk of PCOS.
Study Design: This study included 181 outpatients with PCOS. A total of 301 women who attempted to seek pre-pregnancy consultation at the Department of Gynecology of our hospital were included in the control group, and six coagulation parameters were measured for all included subjects. A logistic regression model was built based on the training dataset using the purposeful selection method to select important predictors. The performance of the established model was validated on the test dataset.
Results: There were statistically significant differences found among all coagulation parameters except D-Dimer (DD, P = 0.080). The purposeful selection method selected age (odds ratio [OR] = 0.89; p = 0.008), prothrombin time (PT, OR = 0.68, p < 0.0001), thrombin time (TT, OR = 3.30; p = 0.0005), and fibrin degradation products (FDP, OR = 0.24; p = 0.0002) as important predictors of PCOS risk. The receiver operating characteristic (ROC) curve analysis indicated that the area under the ROC curve (AUC) of the model was 0.81 for the training dataset with an optimal cut-off point of the predicted probability of 0.45, leading to a sensitivity of 0.71 and a specificity of 0.82. The AUC was 0.79 for the test data.
Conclusions: It was found that the coagulation parameters, including PT, TT, and FDP, are predictive of PCOS. These results highlight the potential of anti-coagulation therapies to lower the risk of adverse outcomes in women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders occurring in women of childbearing age. It is characterized by clinical or biochemical manifestations of excessive androgen, persistent anovulation, and polycystic ovarian changes and often accompanied by insulin resistance (IR) and obesity. In China, the prevalence of PCOS varies based on diagnostic criteria, ethnicity, and region. Based on the 2003 Rotterdam diagnostic criteria, the prevalence of PCOS in Chinese women of childbearing age was approximately 5.6% in 2018 [1]. Furthermore, approximately half of PCOS patients suffer from infertility, with PCOS accounting for as high as 70% of anovulatory infertility [2].

The effects of PCOS on reproduction are not limited to infertility and also include adverse pregnancy outcomes [3,4]. For example, the incidence rate of early and mid-term pregnancy loss in PCOS patients is 20–41% [2], while the incidence rate of PCOS is also higher in women experiencing recurring pregnancy loss (RPL) [5,6]. The exact mechanisms involved in the role of PCOS in infertility and adverse outcomes still need to be further understood. Previous studies have focused on the implications of hyperandrogenaemia, hyperinsulinemia, high levels of luteinizing hormone, and the formation of thrombosis in PCOS patients. Compared with the normal population, PCOS patients exhibit hypercoagulability and low fibrinolysis, which leads to a tendency to develop thrombosis, a major cause of spontaneous abortion [7,8]. When the body is in a prothrombotic state (PTS), the placenta's blood flow decreases. Furthermore, the endometrial blood vessels of the patient become prone to form tiny thrombi, which can lead to the development of a receptive disorder

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that results in embryo implantation difficulties or poorly established post-placental circulation leading to increased plantation failure and embryo loss rates [9,10].

At present, there is no clear examination guide or diagnostic criteria for PTS in patients with PCOS. Thus, this paper aims to analyse coagulation assays in association with PCOS to build and validate a model for the prediction of PCOS risk.

Study design

Study participants

This study included consecutive patients with PCOS treated from January 2017 to August 2018 at the Department of Gynecology of the First People's Hospital of Lianyungang. The data for the control group was taken from outpatients who attempted to seek pre-pregnancy consultation at the Department of Gynecology at this hospital. All subjects in the control group had no observed PCOS and exhibited regular menstruation. Exclusion criteria included: 1) a diagnosis of congenital adrenal hyperplasia, Cushing's syndrome, hypothyroidism, hyperprolactinemia, or other related disorders for the PCOS group and irregular menstruation, signs of hyperandrogenism, or evidence of polycystic ovary (PCO) morphology for the control group; 2) reported pregnancy, thrombotic diseases, malignant tumour history, or pharmacological treatment within 12 weeks of the clinical visit. No subjects took any anticoagulant or procoagulant drugs, contraceptives, or other drugs that could affect sex hormones, insulin, and blood sugar metabolism within three months prior to blood collection, and no subjects ever smoked or drank alcohol.

A total of 219 patients met the Rotterdam criteria as specified below. Of these, 13 patients took oral contraceptives or metformin, 16 patients were excluded due to incomplete medical records, and 9 patients refused to participate. The remaining 181 patients agreed to participate in the study and were included in the analysis. A total of 301 subjects were included in the control group.

This study was approved by the Research Ethics Committee of the First People's Hospital of Lianyungang. Informed consent was obtained from all study participants.

Diagnosis of PCOS

The diagnosis of PCOS was based on the recommendations of the 2003 European Conference on Human Reproduction and Embryology (ESHRE) and the Rotterdam meeting of the American Reproductive Medicine Association (ASRM) [11]: 1) low levels of ovulation or anovulation; 2) clinical manifestations of high androgen (e.g., hirsutism and acne) or hyperandrogenism; and 3) polycystic changes of the ovary $\geq$ 12 follicles with a diameter of 2–9 mm in one or both ovaries and ovarian volume $\geq$ 10 ml. A diagnosis of PCOS could be made if the subject met at least two of the above criteria and did not exhibit any other Kaohsiung diseases, including congenital adrenal hyperplasia, Cushing syndrome, androgen-secreting tumours, or other diseases that cause ovulation disorders, such as hyperprolactinemia, premature ovarian failure, pituitary or hypothalamic amenorrhea, or thyroid dysfunction.

Laboratory analysis

Fasting blood was obtained from all participants upon their first clinical visit or the following day. Data from the coagulation assays, glucose tolerance test, and insulin release test were then collected from all study subjects. The coagulation assays included prothrombin time (PT), activated partial prothrombin time (APTT), thrombin time (TT), fibrinogen (FG), D-Dimer (DD), and fibrin degradation products (FDP). Coagulation data prior to the implementation of any treatment was obtained via the fully automated haemostasis testing system ACL TOP 700 (Instrumentation Laboratory, Bedford, MA, USA) using the following batches: N0972444 (PT), N0278340 (APTT), N0872044 (TT), N0378488 (FIB), B28206 (DD), and B30626 (FDP). Moreover, insulin resistance was calculated using the HOMA-IR formula [12].

Statistical analyses

Continuous data were presented as mean $\pm$ SD and compared using a Wilcoxon rank-sum test. A logistic regression model was built on the training dataset containing 70% of the included subjects using purposeful selection to select important predictors. Compared with traditional variable selection methods for logistic regressions, such as forward selection, backward selection, and stepwise selection, purposeful selection follows a slightly different logic and can select not only significant variables but also important confounders. Simulation studies have indicated that purposeful selection has been superior over existing methods [13]. For purposeful selection, the presence of PCOS acted as the response variable, and variables including age and the coagulation assays were included as candidate variables for variable selection. Moreover, all recommended settings for the inclusion and retention of variables, confounding criteria, and inclusion of noncandidate variables were used.

A receiver operating characteristic (ROC) curve was plotted to evaluate the sensitivity and specificity of the final model. The optimal cut point of the predicted probabilities was determined using the Yuden index, which measures the vertical distance from the uninformative diagonal to the cut point. The performance of the established model was validated on the test dataset.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, USA). $P < 0.05$ was considered to be statistically significant.

Results

Basic characteristics of study participants

A total of 181 PCOS patients and 301 health controls were included in the analyses. The basic characteristics of the study participants are presented in Table 1. Briefly, patients with PCOS were younger ($P = 0.008$), had shorter PT ($P < 0.0001$), activated partial PT ($P = 0.004$), TT ($P < 0.0001$), and larger FDP ($P < 0.0001$). There was also no statistically significant difference in DD ($P = 0.080$).

Association with PCOS

A univariate logistic regression showed that age was not significantly associated with PCOS ($P = 0.054$). In contrast, all six coagulation parameters were significantly associated with PCOS (Table 2). Moreover, the purposeful selection method helped select age (odds ratio [OR] = 0.89; $p = 0.008$), PT (OR = 0.68, $p < 0.0001$), TT (OR = 3.30; $p = 0.0005$) and FDP (OR = 0.24; $p = 0.0002$) as important predictors of PCOS risk (Table 3).

Model discrimination and calibration

The ROC curve is shown in Fig. 1 with a corresponding area under the curve (AUC) of 0.81, indicating that the built regression model has a good discrimination ability. The optimal cut point of the predicted probabilities as determined by the Youden index was $p = 0.45$, which led to a sensitivity of 0.71 and a specificity of 0.82. Fig. 2 is the calibration plot of the predicted model. The calibration

Table 1
Basic characteristics of the study participants.

	PCOS (n = 181)	Control (n = 301)	P
Age	27.33 ± 4.03	28.26 ± 4.03	0.008
BMI[a]	24.98 ± 4.20	–	–
Normal (n, %)	76 (42.0%)	–	–
Overweight (n, %)	65 (35.9%)	–	–
Obesity (n, %)	40 (22.1%)	–	–
Insulin resistance index	3.30 ± 2.32	–	–
Insulin resistance (n, %)[b]	98 (54.8%)		
Prothrombin time (s)	11.43 ± 0.73	12.03 ± 0.72	<0.0001
Activated partial prothrombin time (s)	31.94 ± 3.53	32.72 ± 2.95	0.004
Thrombin time (s)	15.25 ± 1.22	16.01 ± 1.33	<0.0001
Fibrinogen (g/L)	3.03 ± 0.56	2.68 ± 0.40	<0.0001
D-Dimer (mg/L)	75.29 ± 63.27	60.79 ± 40.19	0.080
Fibrin degradation products (μg/mL)	0.68 ± 0.63	0.38 ± 0.33	<0.0001

Data were presented as mean ± SD, and compared using Wilcoxon rank-sum test.
-Data not available.
BMI, body mass index; PCOS, polycystic ovarian syndrome.
[a] Overweight was defined as 24 ≤ BMI<28 and obesity was defined as BMI ≥ 28.
[b] Insulin resistance index was calculated using HOMA-IR and insulin resistance index>2.5 was considered as having insulin resistance. Two patients were excluded due to missing observations of insulin resistance index.

Table 2
Association with PCOS by univariate logistic regression analysis.

	OR (95% CI)	P
Age	0.95 (0.90-1.00)	0.054
Prothrombin time (s)	0.28 (0.19-0.41)	<**0.0001**
Activated partial prothrombin time (s)	0.93 (0.86-1.00)	**0.035**
Thrombin time (s)	0.61 (0.51-0.74)	<**0.0001**
Fibrinogen (g/L)	5.52 (3.20-9.52)	<**0.0001**
D-Dimer (mg/L)	1.01 (1.00-1.01)	**0.005**
Fibrin degradation products (μg/mL)	4.21 (2.45-7.22)	<**0.0001**

P values <0.05 indicate statistical significance and are shown in bold.
CI, confidence interval; OR, odds ratio; PCOS, polycystic ovarian syndrome.

Table 3
Multivariate logistic regression for association with PCOS.

	OR (95% CI)	P
Age	0.89 (0.84-0.95)	**0.0008**
Prothrombin time (s)	0.68 (0.55-0.85)	<**0.0001**
Thrombin time (s)	3.30 (1.76-6.16)	**0.0005**
Fibrin degradation products (μg/mL)	0.24 (0.16-0.37)	**0.0002**

We included variables selected by purposeful selection. INR and PTA were excluded from the candidate list because these two variables were computed and including them might induce multicollinearity.
P values <0.05 indicate statistical significance and are shown in bold.
CI, confidence interval; OR, odds ratio; PCOS, polycystic ovarian syndrome.

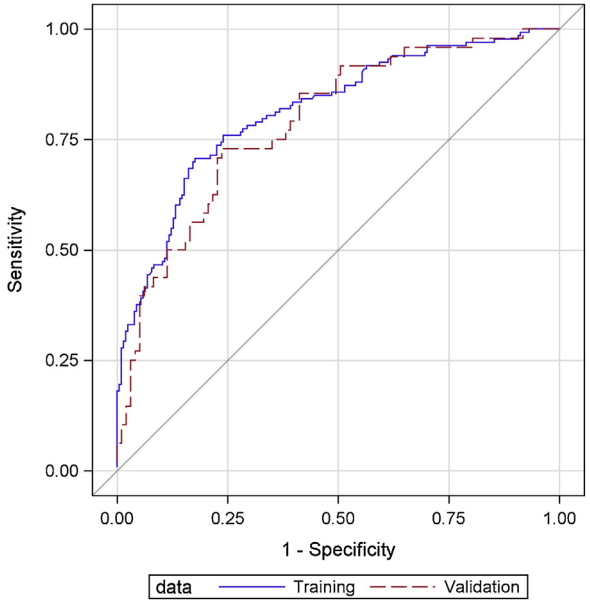

Fig. 1. ROC curve of the prediction model for the training and validation data. AUC is 0.81 for the training data and 0.79 for the validation data.
ROC, receiver operating characteristic; AUC, under the ROC curve.

curve is close to the diagonal reference line, indicating that the predicted and empirical probabilities are similar. This suggests that the built prediction model fits the data well.

Validation of the logistic model

The built logistic regression was applied to the testing data for validation. The ROC curve is shown in Fig. 1 with a corresponding AUC of 0.79 (Fig. 1), indicating a good discrimination ability of the test dataset. With a cut point of 0.45 for the predicted probability, the sensitivity was 0.69 and the specificity 0.77 for the test dataset, which was similar to the performance of the training dataset.

Discussion

This paper examined the coagulation assays in association with PCOS risk and their predictive values, and it was found that PT, TT, and FDP are important predictors of PCOS. Furthermore, the prediction model exhibited a good discrimination and calibration ability for both the training and test data. These analyses reveal the important relationship existing between coagulation assays and PCOS risk and highlight the potential of employing proper anticoagulant therapy in improving reproductive outcomes.

Previous research has reported that the incidence of IR in PCOS patients could be as high as 70% [14]. In this study, it was found that 54.8% of PCOS patients had IR. Insulin resistance and hyperinsulinemia are common characteristics of abnormal glucose metabolism and are closely related to the occurrence of abortion and infertility in patients with PCOS. Moreover, plasminogen activator inhibitor 1 (PAI-1) is a glycoprotein that inhibits fibrinolysis. Hyperinsulinemia induces the up-regulation of PAI-1, leading to the low fibrinolysis of blood [15] and an increased susceptibility to thrombosis, thereby reducing the blood supply during embryo implantation [16]. An increased susceptibility to thrombosis in PCOS patients may also be related to vascular endothelial cell damage and coagulation changes such as

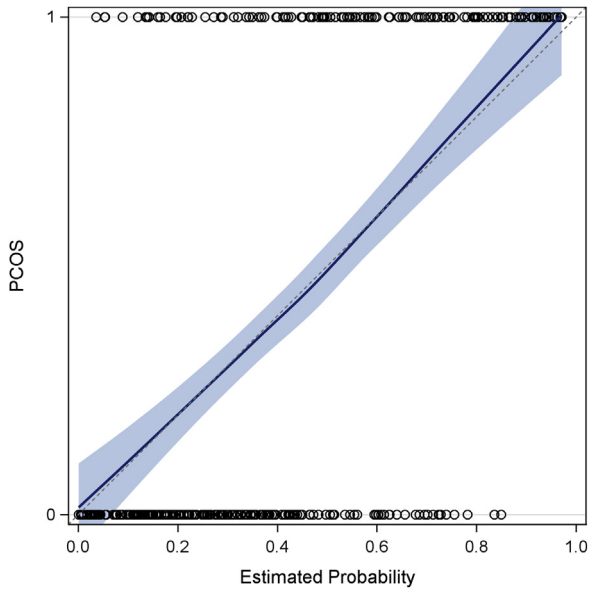

Fig. 2. Calibration plot of the predicted model.
The dotted diagonal line represents the line of perfect calibration. The solid blue line represents the model's predicted probability versus the empirical probability. The blue shaded region represents the corresponding 95% confidence band.
A calibration curve close to the diagonal reference line indicates that the prediction model fits the data well.

hyperglycaemia and hyperinsulinemia. Moreover, IR was reported to be positively correlated with BMI. We found that overweight and obese PCOS patients (i.e., BMI $\geq$ 24) were more likely to have IR than PCOS patients with normal BMIs (i.e., BMI < 24; 75.7% vs. 26.3%; $p < 0.0001$). Unfortunately, due to the retrospective nature of this study, BMI and IR data were not collected from the subjects in the control group, and, therefore, they could not be controlled for in the multivariable logistic regression. Thus, future studies that consider such variables are required to validate our findings.

Patients with PCOS appeared to exhibit a prothrombotic state with altered coagulation and fibrinolysis [17]. Significantly decreased levels of PT, APTT, and TT and significantly elevated levels of FG, DD and FDP were also found in women with PCOS. In this study's prediction model for PCOS, the risk of PCOS increased with decreased levels of PT and TT and elevated levels of FDP. PT also corresponded with extrinsic pathways of coagulation cascade, while a decrease in PT was associated with hypercoagulation [18]. TT was reflected in the *in vivo* anticoagulant, and a shortened TT indicated hypofibrinolysis. With the onset of coagulation, fibrin monomers were transformed by FG into FDP, which reflects overall fibrinolytic activity [18]. Moreover, the combination of PT, TT, and FDP reflects a hypercoagulable state and a disturbance of the haemostatic system. Correspondingly, studies in China and abroad have indicated that adding aspirin to the treatment of PCOS patients could significantly reduce adverse pregnancy outcomes [19,20]. Thus, we are conducting a separate study to compare anticoagulation and non-anticoagulation therapies on their influence on miscarriage rates of PCOS patients. More studies are needed to determine the efficacy of prophylactic prevention of thromboembolism.

Our analysis indicated that younger age is associated with a higher risk of PCOS, implying that PCOS patients might have higher ovarian reserve parameters and an extended window of fertility. However, a recent study suggested that the symptoms of PCOS change over time, and women diagnosed with PCOS at a younger age may fail to meet the Rotterdam criteria once they reach 35–40 years old [21]. These inconsistent findings may indicate certain differences in patient selection, and thus future studies are needed to explore the possible reasons for these differences.

Our study did have certain limitations. The sample size was relatively limited, especially for patients with PCOS. Although purposeful selection was used to include important predictors, this model could have overlooked certain important cofounders, and, therefore, the possibility of residual confounding cannot be ruled out. Furthermore, there could be certain important imbalances arising between the PCOS and the control groups. For example, patients with PCOS were on average younger than the control group (27.3 vs 28.3; $p = 0.008$). Nonetheless, age was controlled for in the multivariable logistic regression, and, therefore, the confounding of age should not be a major concern. Only coagulation parameters were included in the predictive model, therefore, it would be interesting to examine whether coagulation parameters have additive predictive values beyond the traditional risk parameters of PCOS. Unhealthy life styles, long-term use of hormonal drugs, and elevated platelets or haematocrit can also affect the risk of PCOS. Unfortunately, such data were not collected in the present study. Future studies using larger sample sizes to control for such parameters would help further elucidate the effect of coagulation parameters on PCOS risk. More studies are also needed to prospectively collect data and explore whether coagulation assays can be used for the early prediction of PCOS and prophylactic prevention of thromboembolism can be applied to reducing the risk of subsequent adverse pregnancy outcomes.

In conclusion, it was found that several coagulation parameters are predictive of PCOS. These results highlight the potential of anti-coagulation therapies in lowering the risk of adverse outcomes in women with PCOS. Future studies are needed to validate these findings and explore the efficacy of anti-coagulation therapies for PCOS patients.

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References

[1] C.M.D.A.-E.a.M.P. Branch. Endocrine expert consensus on diagnosis and treatment of polycystic ovary syndrome. Chin J Endocrinol Metab 2018;34:1–7.
[2] Yan X, Xu X. Impact of polycystic ovary Syndrome on pregnancy outcome. J Int Reprod Health/Fam Plan 2011;30:371–4.
[3] Palomba S, Falbo A, Russo T, Tolino A, Orio F, Zullo F. Pregnancy in women with polycystic ovary syndrome: the effect of different phenotypes and features on obstetric and neonatal outcomes. Fertil Steril 2010;94:1805–11.
[4] Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update 2006;12:673–83.
[5] Rai R, Backos M, Rushworth F, Regan L. Polycystic ovaries and recurrent miscarriage–a reappraisal. Hum Reprod 2000;15:612–5.
[6] Kazerooni T, Ghaffarpasand F, Asadi N, Dehkhoda Z, Dehghankhalili M, Kazerooni Y. Correlation between thrombophilia and recurrent pregnancy loss in patients with polycystic ovary syndrome: a comparative study. J Chin Med Assoc 2013;76:282–8.
[7] Randeva HS, Tan BK, Weickert MO, Lois K, Nestler JE, Sattar N, et al. Cardiometabolic aspects of the polycystic ovary syndrome. Endocr Rev 2012;33:812–41.
[8] Ghazeeri GS, Nassar AH, Younes Z, Awwad JT. Pregnancy outcomes and the effect of metformin treatment in women with polycystic ovary syndrome: an overview. Acta Obstet Gynecol Scand 2012;91:658–78.

[9] McDonnell R, Hart RJ. Pregnancy-related outcomes for women with polycystic ovary syndrome. Womens Health (Lond) 2017;13:89–97.
[10] Karakurt F, Gumus II, Bavbek N, Kargili A, Koca C, Selcoki Y, et al. Increased thrombin-activatable fibrinolysis inhibitor antigen levels as a clue for prothrombotic state in polycystic ovary syndrome. Gynecol Endocrinol 2008;24:491–7.
[11] Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2013;98:4565–92.
[12] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[13] Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med 2008;3:17.
[14] Stepto NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. Hum Reprod 2013;28:777–84.
[15] Idali F, Zareii S, Mohammad-Zadeh A, Reihany-Sabet F, Akbarzadeh-Pasha Z, Khorram-Khorshid HR, et al. Plasminogen activator inhibitor 1 and methylenetetrahydrofolate reductase gene mutations in iranian women with polycystic ovary syndrome. Am J Reprod Immunol 2012;68:400–7.
[16] Chang EM, Han JE, Seok HH, Lee DR, Yoon TK, Lee WS. Insulin resistance does not affect early embryo development but lowers implantation rate in in vitro maturation-in vitro fertilization-embryo transfer cycle. Clin Endocrinol (Oxf) 2013;79:93–9.
[17] Burchall G, Linden MD, Teede H, Piva TJ. Hemostatic Abnormalities and Relationships to Metabolic and Hormonal Status in Polycystic Ovarian Syndrome. Trends Cardiovascr Med 2011;21:6–14.
[18] Wang T, Kang X, He L, Liu Z, Xu H, Zhao A. Prediction of thrombophilia in patients with unexplained recurrent pregnancy loss using a statistical model. Int J Gynaecol Obstet 2017;138:283–7.
[19] Qiao Y, Zhao K, Wang X. A preliminary study on the effect of aspirin on clinical pregnancy rate in patients with polycystic ovary syndrome. Reprod Contracept 2014;34:838–42.
[20] Chen Y, Leng Q, Xing Q, Xu Y, Zhang Z, Wu J, et al. Effects of low dose aspirin on the pregnancy outcome of PCOS patients during frozen embryo transfer. J Reprod Med 2017;26:14–8.
[21] de Ziegler D, Pirtea P, Fanchin R, Ayoubi JM. Ovarian reserve in polycystic ovary syndrome: more, but for how long? Fertil Steril 2018;109:448–9.